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Lipotropic Activity of Various Compounds Under Standardized Conditions.

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A large number of compounds has been tested for lipotropic activity since the demonstration of the action of choline on liver fat.[1] The laboratories carrying out such studies have used various experimental conditions for demonstrating lipotropic activity. It has often been suggested that the lack of uniformity in conditions has hindered correlation of the data obtained by different investigators. In testing the activity of various substances mice,[2] rats,[3] and depancreatized dogs[4] have been used. The stage of the life cycle of the experimental animal has received little consideration. The diets used for producing fatty infiltration of the liver have ranged from essentially fat-free to very high-fat diets, from protein-free to approximately 25% protein diets.[1] The carbohydrate component has been varied in amount and kind, and dietary mixtures of 2 or 3 different proteins have been utilized.[1] Incomplete vitamin mixtures have been used in special cases.[1] In view of the varying conditions under which different compounds have been reported to be lipotropically active, it seemed important to study a representative group of these compounds under standardized conditions. The substances chosen for study have previously[1] been shown to be lipotropically active.

Experimental. The experimental conditions and techniques were those used in this laboratory in previous studies of lipotropism.[5] Male rats of the Carworth strain weighing approximately 170 g were used. The basal diet contained 15.4% casein, 3.2 % arachin, 5% salt mixture, 2% cellu flour, 34.4% glucose, and 40% lard. This purified choline-free diet has been used extensively in this laboratory in studies of growth and lipotropism. It supplies 500 mg of methionine and 100 mg of cystine per 100 g of diet. Animals of the Carworth strain and of this body weight grew at a slightly slower rate on the basal diet than the strain maintained in this laboratory. Also supplementary choline produces a stimulation of the growth rate in these animals while it fails to do so in our strain.[5] The experimental period was 21 days. All rats received orally 0.1 cc of U.S.P. XI cod liver oil and 0.1 cc of a solution containing 25 γ of thiamine, 20 γ of riboflavin, 100 γ of calcium pantothenate, 100 γ of nicotinic acid and 20 γ of pyridoxine per day. The diets and distilled water were available *ad libitum*. The food intake was determined daily and the weight changes recorded 3 times weekly. The livers were removed from the animals under pentobarbital anesthesia and analyzed for total lipids.[6] In the table the weight changes are recorded as percentage change from the initial weight and liver fat is given in terms of g of fat per 100 g of moist liver, and per 100 g of body weight.

Compounds. With one exception the compounds tested were introduced into the basal diet at the expense of the glucose. The dimethyl sulfide was dissolved in corn oil and injected intraperitoneally each day in an amount of 7 mg per rat. The choline, inositol, betaine, and dimethyl sulfide were commercial products of acceptable purity. The S-methyl-

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2 Eckstein, H. C., and Singal, S. A., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 512.

3 Best, C. H., and Ridont, J. H., *Ann. Rev. Biochem.*, Stanford University, 1938, **7**, 349.

4 Van Prohaska, J., Dragstedt, L. R., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 166.

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6 Tucker, H. F., and Eckstein, H. C., *J. Biol. Chem.*, 1937, **121**, 479.

TABLE I.
Lipotropic Activity of Various Compounds Under Standardized Conditions.

Dietary supplement	%	No. of rats	Change in wt,† %	Food intake per day,† g	Liver lipids per 100 g: Moist liver,† g	Liver lipids per 100 g: Body wt,† g
Basal		10	17.6 ± 2.8	9.1 ± .5	19.1 ± 2.5	1.02 ± .20
Lipociac	0.1	8	17.3 ± 1.7	10.5 ± .4	20.5 ± 2.1	1.06 ± .18
S-ethylcysteine	0.1	7	24.3 ± 2.5	10.2 ± .3	18.6 ± 1.4	.97 ± .11
Cystine betaine	0.1	4	26.4 ± 5.4	11.0 ± .2	17.4 ± 2.8	.92 ± .23
S-methylcysteine	0.1	8	19.8 ± 1.9	10.5 ± .3	17.3 ± 1.0	.91 ± .12
Inositol	0.1	7	26.4 ± 2.9	9.4 ± .2	16.0 ± 1.8	.73 ± .14
Dimethyl sulfide*		8	24.2 ± 3.6	9.7 ± .4	14.8 ± 2.1	.71 ± .20
S-methyl-isothiourea	0.2	7	0.9 ± 3.4	7.0 ± .3	11.5 ± 0.5	.62 ± .04
Betaine	0.1	7	27.9 ± 1.8	8.8 ± .4	12.8 ± 1.1	.52 ± .06
Choline	0.05	8	30.9 ± 5.7	10.3 ± .6	12.3 ± 1.2	.49 ± .07
Choline	0.1	7	30.2 ± 2.5	9.8 ± .5	11.0 ± 1.8	.41 ± .04
Triethyl-choline	0.1	8	23.4 ± 3.6	10.7 ± .4	10.2 ± 1.2	.41 ± .06
Lipociac	1.0	7	39.1 ± 1.9	12.2 ± .3	8.5 ± 0.4	.30 ± .01

* See text

† Including the standard error of the mean calculated as follows: $\sqrt{\Sigma D^2/N - 1}/\sqrt{N}$

isothiourea sulfate,[7] cystine betaine,[8] S-methylcysteine,[9] S-ethylcysteine,[9] and triethylcholine[10] were synthesized in this laboratory and shown by analysis to be of satisfactory purity. The lipociac was prepared from fresh pork pancreas according to the directions of Clark, Eilert and Dragstedt.[11] It contained 9.77% nitrogen, 1.03% methionine, and .92% cystine. The material gave a negative test for choline with ammonium reineckate both before and after hydrolysis.

Results. The results are shown in Table I. They are arranged in order of decreasing liver fat per 100 g body weight. The choline-containing diets were included for comparative purposes. The lipociac (0.1%) and S-ethylcysteine did not influence the level of the liver fat while the S-methylcysteine and the cystine betaine gave small decreases of questionable significance. Inositol, dimethyl sulfide, and S-methylisothiourea sulfate were intermediate in effect. Choline, betaine, triethylcholine, and lipociac (1%) produced the greatest lowering of the liver fat. The amounts of the substances to be incorporated in the diets were chosen to give a submaximum effect so as to eliminate possible side effects of quantities in excess of that utilized in lipotropism. With none of the experimental diets was the liver fat within the normal range (0.15-0.30 g per 100 g body weight) except that containing 1% lipociac. The results with lipociac at the 1% dietary level are especially interesting. Its lipotropic activity was as great or greater than 0.1% choline and in addition it gave the greatest stimulation of growth. The 1% lipociac supplied 1.26 mg of methionine and 1.12 mg of cystine per rat per day. It has been shown that methionine is approximately 1/5 as active as choline in decreasing the liver fat,[1] and the animals receiving choline (0.1%) in this experiment were ingesting 9.8 mg per day which produced a liver fat of 0.41 g per 100 g body weight. Thus it seems quite unlikely that the methionine content of the lipociac was sufficient to account for the lipotropic effect or the stimulation of growth. Moreover, in order to explain the lipotropic activity of the lipociac on the basis of choline as a contaminant, the lipociac would have had to contain approximately 10% choline which certainly would have been detected by the reineckate procedure. These effects of lipociac are being further investigated. The marked depression of the growth rate by S-methyliso-

[7] Shildneck, P. R., and Windus, W., Organic Synthesis, New York, 1943, Coll., **2**, 411.

[8] Schubert, M. P., *J. Biol. Chem.*, 1935, **111**, 671.

[9] du Vigneaud, V., Loring, H. S., and Craft, H. A., *J. Biol. Chem.*, 1934, **105**, 481.

[10] Channon, H. J., and Smith, J. H. B., *Biochem. J.*, 1936, **30**, 115.

[11] Clark, D. E., Eilert, M. L., and Dragstedt, L. R., *Am. J. Physiol.*, 1945, **144**, 620.

thiourea sulfate is also of interest and suggests that further investigation with this compound might be profitable. It is possible that the effect of S-methylisothiourea sulfate on growth is due to the phenomenon of metabolic antagonism.

Summary. A representative group of substances, previously reported to be lipotropically active, have been tested under standardized conditions. Under the present experimental conditions and in the amounts fed, S-ethylcysteine did not decrease the level of the liver lipids, cystine betaine and S-methylcysteine exhibited a slight activity of questionable significance, inositol, dimethyl sulfide and S-methylisothiourea sulfate were of intermediate activity, and betaine and triethylcholine had approximately the activity of choline. Lipociac, at the 1% dietary level, was highly active lipotropically and stimulated growth to a greater degree than any of the other compounds tested. S-methylisothiourea sulfate depressed the growth rate.

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Effects of Temperature and Ultraviolet Light on Experimental Polyarthritis of Rats.*

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The experimental polyarthritis produced by the L-4 strain of pleuropneumonia-like microbes in rats is not strictly comparable to rheumatoid arthritis in man. However, because it responds to gold therapy similarly to rheumatoid arthritis, we consider it a method for studies of factors which might influence the arthritic processes and for making chemotherapeutic trials in animals.

In this study attempts were made to determine whether changes in environmental temperature or in ultraviolet irradiation would alter the course of the disease. This is of importance since, in man, it has commonly been supposed that changes in temperature and ultraviolet irradiation would alter the course of rheumatoid arthritis and the incidence of rheumatic fever. Therefore, we decided to evaluate the effects of these environmental changes with the object of increasing basic knowledge regarding the natural history of the disease in the rat.

Exposure. Thirty male and 30 female albino rats weighing 100 g were placed in an outdoor shelter with open screen sides which protected them from wind, rain and sunshine but not from changes in temperature. The nocturnal temperature ranged from 30 to 45°F, mean 37°F; the diurnal temperature ranged from 46 to 84°F, mean 54°F, the overall range being 30 to 84°F, mean 45°F. The temperature records were made on an automatic thermograph. The average relative humidity was 86% at 5 A.M., 64% at noon, and 70% at 5 P.M.‡ The diet used was Purina Dog Checkers (compressed pellets containing protein 21%; fat 4%; fibre 6%; nitrogen-free extract 46%; and ash 9%). All animals were allowed free access to water. For one week prior to inoculation with pleuropneumonia-like organisms (P.L.O.§) the animals were subjected to exposure. They were then given intraperitoneally 2 cc of a

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‡ Official figures supplied by U. S. Weather Bureau, San Francisco.

§ Abbreviation used throughout to designate L_4 strain of pleuropneumonia-like organisms.